Preparation of DNA probes for chromosome FISH: Whole chromosome painting probes labeling by DOP-PCR

Reagents and equipment

- Source DNA (flow sorted or microdisected chromosomes)
- PCR buffer 10X Perkin Elmer without $MgCl_2$ (Roche Molecular Biochemical)
- $MgCl_2$ solution 25 mM Perkin Elmer (Roche Molecular Biochemical)
- Taq DNA polymerase 5U/μl Perkin Elmer (Roche Molecular Biochemical)
- Deoxy-nucleotides dATP, dCTP, dGTP, and dTTP 100 mM (Roche Molecular Biochemical)
- Stock dNTPs solution for labeling PCR contains 0.2 mM of dATP, dCTP, dGTP and 0.15 mM of dTTP
- 0.06mM Fluorescein–dUTP. (Boehringer Mannheim) or Biotin-16-dUTP (Boehringer Mannheim) or Spectrum red-dUTP (Vysis)
- Universal primer for human genomic DNA amplification: UN1 (Midland Certified Reagent Co) Telenius [5'-CCGACTCGAGNNNNNNATGTGG-3'] or universal primer for mouse genomic DNA amplification: 22-mer (Midland Certified Reagent Co.) [5'-CGG ACT CGA GNN NNN NTA CAC C-3']
- Hi-Lo DNA marker (Minnesota Molecular)
- Tris acetate buffer
- Sodium Acetate 3M pH 5.2
- PCR thermocycler
- Microcentrifuge
- Microcentrifuge tubes

Method

1. Set up the PCR reaction.

PCR reaction mix:

Reagent	Quantity (μl)

PCR buffer 10X	10
$MgCl_2$ 25mM	8
Stock dNTPs solution[a]	5
Biotin–dUTP[a]	5
dH_2O	65
DNA (100-150 ηg/μl)	4
Primer (100 μM)	2
Taq polymerase (5U/μl)	1

2. Run the PCR reaction

Step	Temperature (°C)	Time (min)
1	94	1
2	56	1
3	72	3 (+1 additional sec/cycle)
4	Steps 1-3 (29 times)	
5	72	10

After the final step hold the PCR samples at 4°C until they are used.

3. To analyze the DOP-PCR products, mix 8 μl of the reaction products with 2 μl agarose gel loading buffer.
4. Apply the sample to a 1% w/v agarose gel in 1x TAE buffer.
5. Apply 10 μl of Hi-Lo DNA marker.
6. Run the gel for 45 min at 70 V/cm in 1x TAE buffer.
7. Stain the gel with ethidium bromide and observe in UV transiluminator. In the reaction samples you should see a smear of DNA ranging from about 200–500 bp.
8. Add to the remnant of the DOP-PCR labeled DNA 1/10 volume of 3M sodium acetate pH 5.2 and 3 volumes of cold absolute ethanol.
9. Put tubes at -70 °C for 30 min.

10. Spin down the samples at 14,000 g in 4 °C microcentrifuge for 30 min.
11. Remove carefully the supernatant and dry the DNA under vacuum for 3 min.
12. Resuspend the DNA in sterile water at a final concentration of 100 ηg/μl.
13. Store the labeled DNA at –20 °C.

[a]Note that the concentration of the dNTPs and labeled-dUTP varies.

- Remnants of cytoplasm can impair the access of the probe to the target DNA of metaphase and especially interphase chromosomes. To improve the hybridization quality a treatment with 70% acetic acid and/or mild pepsin treatment is frequently performed. Here is the protocol

Equipment and reagents

- Glacial acetic acid
- Hydrochloric acid 1N
- Pepsin (Sigma)
- 1XPBS
- Coplin jars
- Slide warmer or waterbath

Method for acetic acid treatment

1. Soak the slide in an acetic acid 70 % v/v solution for 40-60 sec.
2. Rinse the slide in 1xPBS for 5 min at room temperature, shaking gently.
3. Dehydrate the slide trough an ethanol series of 70 % v/v, 85% v/v, and 100 % v/v for 2 min each at room temperature and then air dry.

Method for pepsin treatment

1. Rinse the slide in 1XPBS for 5 min.

2. Apply to a 60 mm^2 coverslip 100 µl of a 10µg/ml solution of pepsin in 10 mM HCl. Touch the slide to the coverslip. Incubate in moist chamber at 37°C, for 5 min.
3. Rinse the slide in 1XPBS for 5 min.
4. Dehydrate the slide trough an ethanol series of 70 % v/v, 85% v/v, and 100 % v/v for 2 min each at room temperature and then air dry.